ECTOTROPHIC MYCORRHIZAE AS BIOLOGICAL DETERRENTS TO PATHOGENIC ROOT INFECTIONS BY PHYTOPHTHORA CINNAMOMI

Dr. Donald H. Marx, Plant Pathologist
Southeastern Forest Experiment Station
Forestry Sciences Laboratory
Carlton Street, Athens, Georgia, U.S.A.

Numerous investigators in the last several decades have shown that ectotrophic mycorrhizae are important in the establishment and growth of trees. Their beneficial effect, proved experimentally, is of a direct physiological nature; i.e., increased root absorption surface, selective ion absorption and accumulation, ability to render unavailable substances in soil available to the plant host, etc.

In addition to the direct physiological advantages, another benefit of mycorrhizae to plants has been discussed but not tested experimentally. In 1964, Dr. Bratislav Zak proposed that fungus symbionts can protect delicate, unsuberized root tissues from attack by pathogenic fungi. He suggested that mycorrhizal fungi may protect these succulent root tissues by: (a) utilizing surplus root carbohydrates and thus reducing the attractiveness of the root to pathogens; (b) serving as a physical barrier to infection; (c) secreting antibiotics; and (d) favoring, along with the root, protective rhizosphere organisms.

The purpose of this report is to present recent results of research aimed at testing the root protection hypothesis.

The major test root pathogen was Phytophthora cinnamomi Rands, the causal organism of littleleaf disease of shortleaf (Pinus echinata Mill.) and loblolly (P. taeda L.) pines. This fungal pathogen was selected because it

infects the same root tissue invaded by mycorrhizal fungi; it initiates infection by motile zoospores which are chemically attracted to roots of host plants, thus allowing for tests on the chemical attractiveness of mycorrhizae; and its hosts include shortleaf and loblolly pines which are also hosts for several known mycorrhizal fungi.

In the initial research, various mycorrhizal fungi were screened for antibiotic production by using agar plate antagonism tests against numerous taxonomically different fungal root pathogens. Results indicated a wide range of antibiotic production, from 92% to 0%, for the symbionts (Table 1). Based on the data, it appeared that each antagonistic symbiont produced an antibiotic with a different biological activity since certain pathogens were inhibited by one symbiont but not by certain others. The major antibiotic produced by Leucopaxillus cerealis var. piceina was spectrophotometrically identified as diatretyne nitrile (HOOC-CH=CH-C≡C-C≡C-C≡N). The symbiont also produced two other polyacetylenic antibiotics, diatretyne amide (HOOC-CH=CH-C≡C-C≡C-$CONH_2$) and diatretyne 3 (HOOC-CH=CH-C≡C-C≡C-C≡C-CH_2OH), which are antibacterial in action. Diatretyne nitrile inhibited germination of zoospores of P. cinnamomi at concentrations of 50 parts per billion and was lethal to zoospores at 2 parts per million (ppm). The antibiotic also inhibited soil bacteria at 0.5 to 2.5 ppm; it did not inhibit aseptic germination of shortleaf pine seed after 2 hours' exposure at concentrations up to 40 ppm, but it was phytotoxic to aseptic shortleaf pine seedling growth at 8 to 10 ppm. Leucopaxillus cerealis var. piceina produced maximum quantities (12 ppm) of diatretyne nitrile during its rapid growth phase in liquid culture. Diatretyne amide and diatretyne 3 were present in greatest quantities during the autolytic growth phase. Diatretyne nitrile was autoenzymatically removed from culture by the symbiont during the autolytic growth phase.

The diatretyne antibiotics were produced by L. cerealis var. piceina in all media which would support growth. These included a variety of agar and liquid synthetic media, shortleaf pine root extract, sterile forest

Table 1

Antagonism of five ectotrophic mycorrhizal fungi to a variety of root pathogenic fungi in agar medium

Test Pathogen	Laccaria laccata	Lactarius deliciosus	Leucopaxillus cerealis var. piceina	Pisolithus tinctorius	Suillus luteus
Armillaria mellea	-	-	-	-	-
Cylindrocladium scoparium	-	-	+	-	+
Fomes annosus	-	-	+	-	-
Fusarium oxysporum var. pini	-	-	-	-	+
Phytophthora spp. (9)	+ 11% - 89%	+ 83% - 17%	+ 100% - 0%	+ 0% - 100%	+ 100% - 0%
Polyporus tomentosus var. circinatus	-	+	+	-	-
Poria weirii	-	-	+	-	+
Pythium spp. (24)	+ 67% - 33%	+ 0% - 100%	+ 100% - 0%	+ 0% - 100%	+ 92% - 8%
Rhizoctonia spp. (8)	+ 0% - 100%	+ 13% - 87%	+ 75% - 25%	+ 0% - 100%	+ 25% - 75%
Sclerotium bataticola	+	-	+	-	-
% Pathogens Inhibited	35%	16%	92%	0%	76%

Overall % pathogen inhibition by all mycorrhizal fungi 44% (101 of 231)

humus with sucrose or malt extract supplements, and vermiculite-peat moss mixtures of different pH values moistened with Melin-Norkrans synthetic nutrient. The symbiont formed mycorrhizae containing diatretyne nitrile and diatretyne 3 on roots of shortleaf pine in aseptic culture. Both antibiotics were also detected in the rhizosphere of the mycorrhizae.

A special technique was devised for inserting individual root segments (i.e., lateral roots supporting non-mycorrhizal short roots, mycorrhizae, and lateral root tips) of intact seedlings into small glass cylinders. Zoospores of P. cinnamomi were placed in the glass cylinders and the remainder of the root system not sealed in cylinders was covered with sterile sand moistened with inorganic nutrients. This technique permitted, during pathogen incubation, microscopic examination of individual root segments during the infection process while the remainder of the root system was maintained in good physiological condition. This technique was used to inoculate non-mycorrhizal feeder roots and mycorrhizae of shortleaf and loblolly pine seedlings formed aseptically by Laccaria laccata, Leucopaxillus cerealis var. piceina, Pisolithus tinctorius, and non-mycorrhizal short and lateral roots and mycorrhizae of different morphological types (symbionts unknown) on potted shortleaf pine seedlings grown in the greenhouse.

Histological examination showed that mycorrhizae with complete mantle and Hartig net development were totally resistant to infection from zoospores of P. cinnamomi (Table 2 and 3). In addition, non-mycorrhizal short roots adjacent to mycorrhizae formed by the diatretyne nitrile-producing fungus, L. cerealis var. piceina, were only 25% as susceptible as other short roots, indicating that they may have been protected by the antibiotic. Certain mycorrhizae with incomplete mantle development, i.e., a few mycorrhizae formed by P. tinctorius on shortleaf and loblolly and by S. luteus on loblolly pine seedlings, were infected by the pathogen. Spread of infection, however, in the mycorrhizal root cortex stopped at regions of Hartig net development in all instances. Short root initials, not fully

Table 2

Phytophthora cinnamomi infection of roots of aseptic shortleaf pine seedlings with and without mycorrhizae

Mycorrhizal fungus	No. seedlings	Inoculation with zoospores of *Phytophthora cinnamomi*					
		Mycorrhizae		Short roots		Lateral root tips	
		No. inoculated	% infected	No. inoculated	% infected	No. inoculated	% infected
Laccaria laccata	9	7	0	29	100	8	100
Leucopaxillus cerealis var. *piceina*	6	27	0	32	25	6	100
Pisolithus tinctorius	9	42	19[a]	13	100	6	100
Suillus luteus	8	9	0	34	77	6	100
Non-mycorrhizal control	7	--	--	23	100	8	100

[a]Mycorrhizae with incomplete mantle and Hartig net development.

Table 3

Phytophthora cinnamomi infection of roots of aseptic loblolly pine seedlings with and without mycorrhizae

Mycorrhizal fungus	No. seedlings	Inoculation with zoospores of Phytophthora cinnamomi					
		Mycorrhizae		Short roots		Lateral root tips	
		No. inoculated	% infected	No. inoculated	% infected	No. inoculated	% infected
Laccaria laccata	8	8	0	9	100	4	100
Pisolithus tinctorius	9	29	21[a]	16	100	4	100
Suillus luteus	7	12	17[a]	47	85	6	100
Non-mycorrhizal controls	7	--	--	36	100	8	100

[a]Mycorrhizae with incomplete mantle and Hartig net development.

differentiated, covered by fungus mantles from adjacent mycorrhizae on the lateral root were not infected by the pathogen, whereas all initials not covered by mantles were heavily infected.

Similar results were obtained from feeder roots of potted shortleaf pine seedlings (Table 4). All mycorrhizae, regardless of morphological type, were resistant to infection by P. cinnamomi as long as the mantle coverings were complete. Certain mycorrhizae without complete mantle coverings were infected by the pathogen but the infection did not spread into regions of Hartig net development.

To test further the influence of the Hartig net on spread of infection by P. cinnamomi, mycorrhizal and non-mycorrhizal short and lateral root tips were detached from potted shortleaf seedlings and excised at the extending tips with a scalpel. Histological examination after incubation with P. cinnamomi revealed that all non-mycorrhizal short and lateral root tips were heavily infected by the pathogen. However, infection of mycorrhizae was only evident in the stelar tissues and was completely absent from cortical cells surrounded by the Hartig net (Table 5).

Chemotaxis of zoospores of P. cinnamomi was not observed toward intact mycorrhizae or non-mycorrhizal feeder roots of shortleaf or loblolly pines, but attraction of zoospores was observed toward the stelar tissues of all roots with their extending tips excised.

The conclusion drawn from these results is that complete ectotrophic mycorrhizae of shortleaf and loblolly pine seedlings are resistant to infection by P. cinnamomi. The precise mechanism by which they are resistant is difficult to elucidate. It does appear, however, that the fungus mantle functions as a barrier to infection, because short root initials not infected by a mycorrhizal fungus but passively covered by the fungus mantle were resistant to infection. It also appears that the Hartig net is either a physical or chemical barrier because infection by the pathogen did not spread into these regions. Protection of mycorrhizae by symbiont antibiotic production is still not clear because the presence of fungus mantle was

Table 4

Phytophthora cinnamomi infection of roots of shortleaf pine seedlings grown in non-sterilized humus in greenhouse pot culture

Root type	Infection by *Phytophthora cinnamomi*			
	3 days after inoculation		10 days after inoculation	
	No. inoculated	% infected	No. inoculated	% infected
Mycorrhizal form 1	12	25[a]	14	43[a]
Mycorrhizal form 2	17	0	7	0
Mycorrhizal form 3	23	0	11	0
Non-mycorrhizal short root	16	100	11	100
Non-mycorrhizal lateral root tip	6	100	6	100

[a]Mycorrhizae with incomplete fungus mantle and Hartig net development.

Table 5

Phytophthora cinnamomi infection of detached and modified roots of shortleaf pine seedlings grown in autoclaved humus in greenhouse pot cultures

Treatment	Infection of indicated root type						
	Mycorrhizae			Short roots		Lateral root tips	
	Complete	Cortex exposed	Stele exposed	Complete	Tips removed	Complete	Tips removed
Inoculated	7	9	11	5	13	5	5
Infected	0%	0%	100%[a/]	100%[b/]	100%[b/]	100%[b/]	100%[b/]

a/Stelar tissue infected only.

b/Cortex and stelar tissues infected.

sufficient to insure resistance to infection. However, antibiotic production appeared to have significance in rendering adjacent non-mycorrhizal short roots resistant.

Current research is underway to determine the resistance of mycorrhizae to infection by other root pathogenic fungi. Screening various ectotrophic mycorrhizal fungi for antibiotic production is also continuing. Major emphasis is being placed on testing the root protection concept against different root pathogens in greenhouse, nursery, and in field plots with known mycorrhizal associations on roots of different pine species.